Anat Embryol (1992) 185:101–113

Anatomy and Embryology

Review article

The vertebrate tail bud: three germ layers from one tissue

C. May Griffith[1], M.J. Wiley[2], and Esmond J. Sanders[3]

[1] Department of Anatomy and Cellular Biology, Harvard Medical School, 220 Longwood Avenue, Boston, MA 02115, USA
[2] Department of Anatomy, University of Toronto, Toronto, Ontario, M5S 1A8, Canada
[3] Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

Accepted August 20, 1991

Summary. The tail bud of amniote embryos comprises a mass of apparently undifferentiated mesenchymal cells located at the caudal limit of the embryo, representing the remains of Hensen's node and the primitive streak. These cells have the potential to give rise to a variety of different tissues including the posterior or 'secondary' neural tube, the tail gut, and somites and their derivatives. This seemingly homogeneous accumulation of cells therefore has the capacity to differentiate into tissues which in more cranial regions of the embryo are derived from cells of different germ layers. In this review, the tissue contributions of the tail bud in various vertebrate classes are discussed, with particular attention to the mesenchymal-to-epithelial transformation that characterizes the process of secondary neurulation, and which distinguishes it from the epithelial folding that occurs during primary neurulation in more cranial regions. Recent studies suggest that the transformation is accompanied by extensive changes in the cell surface oligosaccharide complement of the differentiating cells, and that the sialyted form of N-CAM is expressed both temporally and spatially in a manner that suggests a role for it in the process. The pluripotential nature of the tail bud mesenchyme may be revealed experimentally by grafting the tissue ectopically, or by culturing it on different substrata. In the latter case, the mesenchyme can be demonstrated to give rise to myocytes, chondrocytes, neuroepithelium and neural crest derivatives such as melanocytes, depending on the nature of the culture substratum. It is concluded that the tail bud mesenchyme represents a developing system which is readily amenable to experimentation and should provide insights into the general mechanisms of cell differentiation and transformation.

Key words: Tail bud – Germ layers – Vertebrate embryos – Differentiation

Introduction

Holmdahl (1925 a, b, c) first hypothesized that the development of the body of vertebrate embryos occurs in two distinct and separate phases. The first phase, primary body development, 'primärer Körperentwicklung', results in the establishment of the three classical germ layers of embryology through the process of gastrulation. The early amniote embryo is a two-layered structure, comprising an epiblast and an endoblast. During gastrulation, epiblast cells invaginate at the midline to form a primitive streak. Involution of cells through the primitive streak gives rise to the mesoderm. The anterior and greater portions of axial organs such as the neural tube, notochord and primitive gut, are examples of 'primary body-derived' structures.

Subsequent to 'primary body development', secondary body development, 'sekundärer Körperentwicklung', occurs with the formation of an undifferentiated masss of mesenchymal cells, the tail bud (or end bud), at the caudal limit of the embryo. The tail bud gives rise to the secondary portion of the body, which includes caudal axial structures such as the caudal or secondary neural tube, tail gut and possibly the notochord. It also forms the caudal-most somitic derivatives: the vertebral column and associated musculature. These tissues in 'primary body-derived' regions would have arisen from all three germ layers.

In this review, the differentiation of the tail bud in different vertebrate classes and its contribution towards various caudal structures of the body, particularly the caudal or secondary neural tube will be discussed. We will also examine what is known about the factors that affect the differentiation of the tail bud, focusing mainly on the macromolecules of the cell surface and extracellular matrix, as these are known to have profound effects on cellular differentiation during embryogenesis.

Development of the tail bud: secondary neurulation

The tail bud is an aggregate of mesenchymal cells located at the caudal limit of vertebrate embryos, just distal to the location of the posterior neuropore (the last part of the neural folds to undergo midline fusion). In birds and in mammals at least, these mesenchymal cells represent the coalescence between the remains of Hensen's node and the remains of the primitive streak at the end of gastrulation.

Offprint requests to: E.J. Sanders

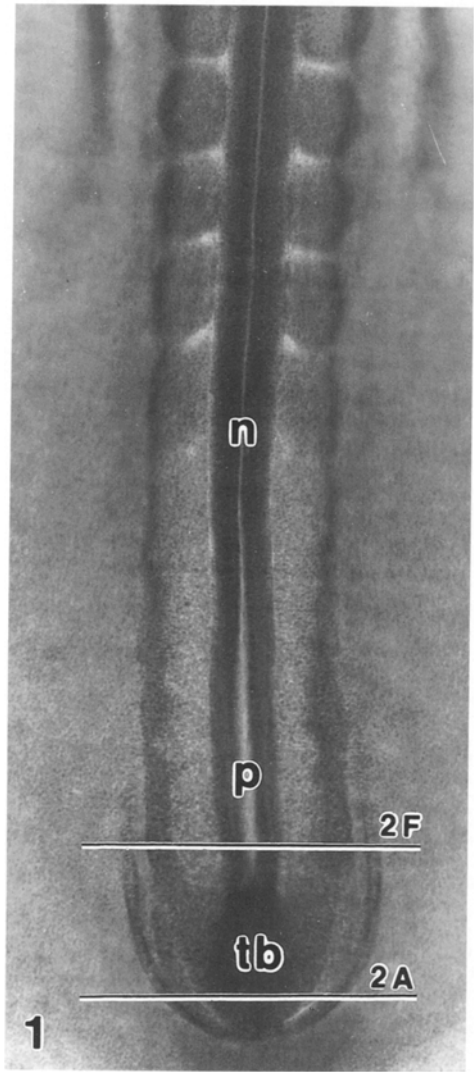


Fig. 1. Caudal region of HH-stage 15 chick embryo, showing the position of the tail bud at the caudal limit of the closing posterior neuropore (*p*). Serial sections were taken in a caudal to cranial sequence (*2A* to *2F*). As secondary neurulation proceeds in a cranial to caudal fashion, these sections (shown in Fig. 2A–F) represent increasingly advanced stages in tail bud differentiation. *tb*, tail bud; *n*, primary neural tube. ×70 (modified with permission from Schoenwolf 1979)

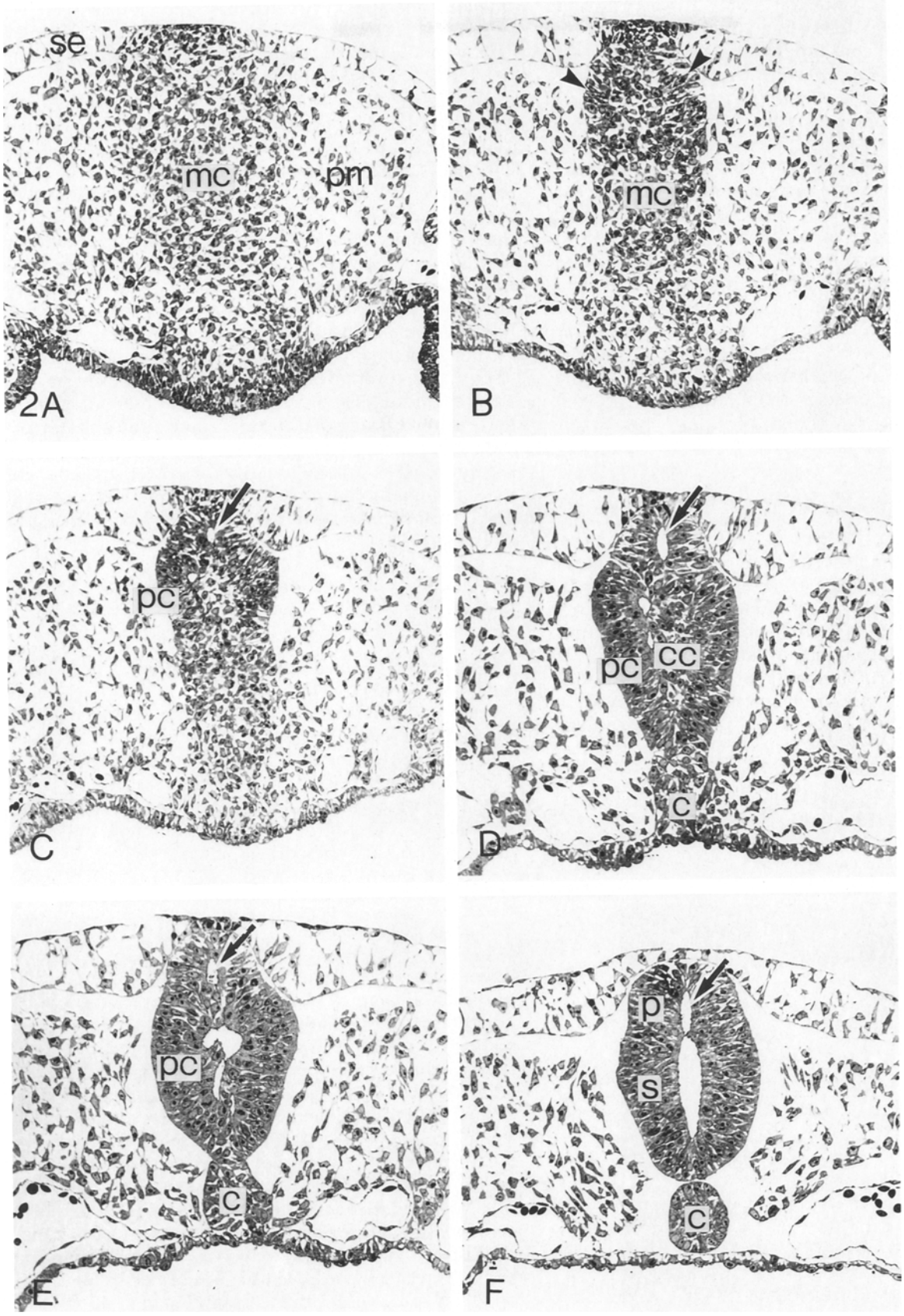
In most vertebrates, the cranial portion of the neural tube, which will form the brain and most of the spinal cord, is derived from the neural plate that forms after gastrulation, through a process called primary neurulation. Primary neurulation involves the bending of the neural plate, which results in the formation of the neural groove and flanking neural folds. The neural folds then appose and fuse across the dorsal midline to form the primary neural tube (Karfunkel 1974; Schoenwolf and Smith 1990). Subsequent to primary neurulation, the caudal or secondary neural tube develops by the cavitation of a solid mass of cells, the tail bud, to form a neurocoele.

Avians

The development of the tail bud has been most extensively studied in the chick embryo. The tail bud begins to form at approximately stage 11 according to the criteria of Hamburger and Hamilton (1951), through the consolidation of the remnants of Hensen's node and the primitive streak (Schoenwolf 1979; Schoenwolf and DeLongo 1980; Schoenwolf and Smith 1990). At stages 13–14, the tail bud is an aggregate of cells at the caudal limit of the embryo (Fig. 1). Differentiation proceeds in a cranial to caudal direction, beginning with the formation of a solid midline cord of cells within the tail bud, the medullary cord, flanked by paraxial mesoderm on either side (Fig. 2A). Subsequently, the cells of the medullary cord become divided into two populations (Fig. 2B). The peripherally situated population undergoes a mesenchymal to epithelial transformation to form a primitive neuroepithelium; while the centrally placed cells retain their mesenchymal characteristics. Cavities begin to form at the boundary between the two cells populations (Fig. 2C; Schoenwolf 1979; Schoenwolf and DeLongo 1980; Schoenwolf and Smith 1990). These cavities enlarge while the central cells merge with the peripheral cells, presumably by intercalation (Fig. 2D–F). Further enlargement and subsequent coalescence of the cavities result in a single lumen which becomes continuous with the neurocoele of the primary neural tube (Criley 1969; Klika and Jelinek 1969; Hughes and Freeman 1974; Schoenwolf 1977, 1978, 1979; Schoenwolf and DeLongo 1980).

There is an added complication to the development of the caudal levels of the neuraxis in chick embryos. At the level of the posterior neuropore, there is a transitional or overlap zone, within which both primary and secondary mechanisms of neurulation operate (Schoenwolf 1979; Schoenwolf and DeLongo 1980; Dryden 1980). Dryden (1980) has described the morphology of this zone as a double wedge. The dorsal aspect of this zone is formed by the neural folds of the posterior neuropore that lie above the medullary cord and tapers caudally. The underlying medullary cord in turn tapers

Fig. 2A–F. Transverse sections through the tail bud and overlap zone of a HH-stage 15 chick embryo, arranged in a caudal to cranial sequence. ×180. **A** Section through the tail bud, showing the solid medullary cord (*mc*), flanked by paraxial mesoderm (*pm*) on either side. *se*, surface ectoderm. **B** Differentiation of the medullary cord begins with the formation of two cell populations. The *arrowheads* indicate mesenchymal cells that have undergone transformation to form elongated primitive neuroectodermal cells. **C** The transformed cells are found in the peripheral area (*pc*). The centrally-located cells retain their mesenchymal characteristics. The *arrow* in this and subsequent panels indicates the caudal end of the primary neural tube lumen. **D** Cavitating medullary cord. Several lumina are shown at the boundary between the peripheral cells (*pc*) and central cells (*cc*). *c*, notochord. **E** Coalescence of lumina and integration of central cells into the peripheral population. **F** Late cavitation in the overlap zone, showing almost complete fusion between the primary (*p*) and secondary (*s*) neural tubes (modified with permission from Schoenwolf and DeLongo 1980)


se
mc
pm
2A
mc
B
pc
C
pc
cc
c
D
pc
c
E
p
s
c
F

cranially, ventral to the neural folds. This overlap zone gives rise to the lower lumbar and upper sacral segments of the future spinal cord (approximately somites 28–34; Constanzo et al. 1982), while the lower sacral and coccygeal cord levels are derived entirely from the tail bud.

Since the development of the lumbosacral levels of the neural tube involves a very close spatial and temporal relationship between primary and secondary mechanisms, and since pressure generated and exerted by the fluid contained within the neurocoele is important in primary neurulation, especially in the morphogenesis of the brain, it has been suggested that subsequent to posterior neuropore closure, pressure generated by this 'neural tube fluid' could initiate cavitation by erosion of the medullary cord (Coulombre and Coulombre 1958; Desmond and Jacobson 1977). However, Constanzo et al. (1982) have shown that this probably is not the case, and that development of the tail bud appears to be autonomous.

Mammals

In most mammalian species studied, the development of the tail bud is simpler than that in chicks. In rodents, the tail bud is an aggregate of mesenchymal cells located at the tip of the tail (Fig. 3) after closure of the posterior neuropore. It is present at $10^1/_2$ to 11 days of gestation (day of vaginal plug found in dam after mating = day 1). There is no formation of multiple cavities during the course of its development. Instead, the lumen of the primary neural tube extends caudally into the medullary cord symmetrically, while the mesenchymal cells of the medullary cord become radially arranged around it (Fig. 4). Subsequently, the medullary cord cells surrounding the lumen undergo a mesenchymal to epithelial transformation to form the secondary neural tube (rat, mouse, opossum, pig: Hughes and Freeman 1974; mouse: Schoenwolf 1984; hamster: Shedden and Wiley 1987). As in the chick embryo, the sequence of events proceeds in a cranial to caudal direction.

The development of the tail bud in humans is still controversial. According to Lemire (1969) and Hughes and Freeman (1974), who examined serial sections through human embryos and reported the presence of cavities within the tail bud, cavitation of the tail bud occurs in a manner similar to that in chickens. Müller and O'Rahilly (1987), however, did not find multiple cavities in their specimens and therefore, argued that secondary neurulation in humans involves the gradual and even extension of the primary neurocoele into the tail bud, in a process which resembles that described in other mammalian species. They further argued that there is no overlap zone of neurulation such as that seen in chicks. The discrepancy in the observations may have arisen because of differences in fixation and/or fixation artifacts that were present in the different specimens examined. It is generally agreed, however, that the tail bud in humans contributes to the sacral and coccygeal levels of the spinal cord (Lemire et al. 1975; Müller and O'Rahilly 1987); cord levels similar to those in chicks.

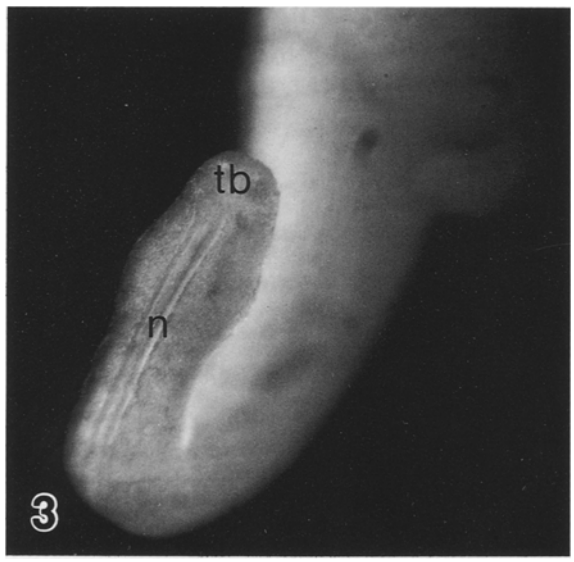


Fig. 3. The tail region of a $11^1/_2$ day mouse embryo. The tail bud (*tb*) is located at the caudal end of the completely closed primary neural tube (*n*). ×40 (from Schoenwolf 1984, with permission)

Amphibians

Development of the amphibian tail bud has been most extensively studied in the primitive amphibians, the urodeles. In amphibians, however, what is described as the tail bud is the caudal extension of more cranial structures that give rise to the tail, which is a large part of the embryo (Deuchar 1975; Jacobson 1988). There is no caudally located aggregate of mesenchymal cells corresponding to those in amniotes that differentiate subsequent to 'primary body development'.

Lampreys (Cyclostomes)

According to Nakao and Ishizawa (1984), the tail bud of the larval lamprey (*Lampetra japonica*) comprises two adjacent cell populations (C1 and C2), arranged in a cranial-to-caudal fashion. The caudal population (C1) is a loose aggregate of polymorphous cells that extends cranially as a sheet over the anterior group (C2). This cellular sheet subsequently differentiates into somites. The C2 cell population is a compact mass of horizontally-stacked cells that are elongated mediolaterally. The upper one-third of these cells develops into the neural tube; the middle one-third differentiates into the notochord; while the remaining cells give rise to two other chord-like structures, referred to as the subchord, and an undefined cell cord. Neurulation begins in the upper one-third of C2 cells with the formation of multiple cavities. As in chicks, these cavities enlarge and coalesce to form a neurocoele or central canal. Simultaneously, the C2 cells become radially rearranged around this developing lumen, and acquire apicobasal polarity to form

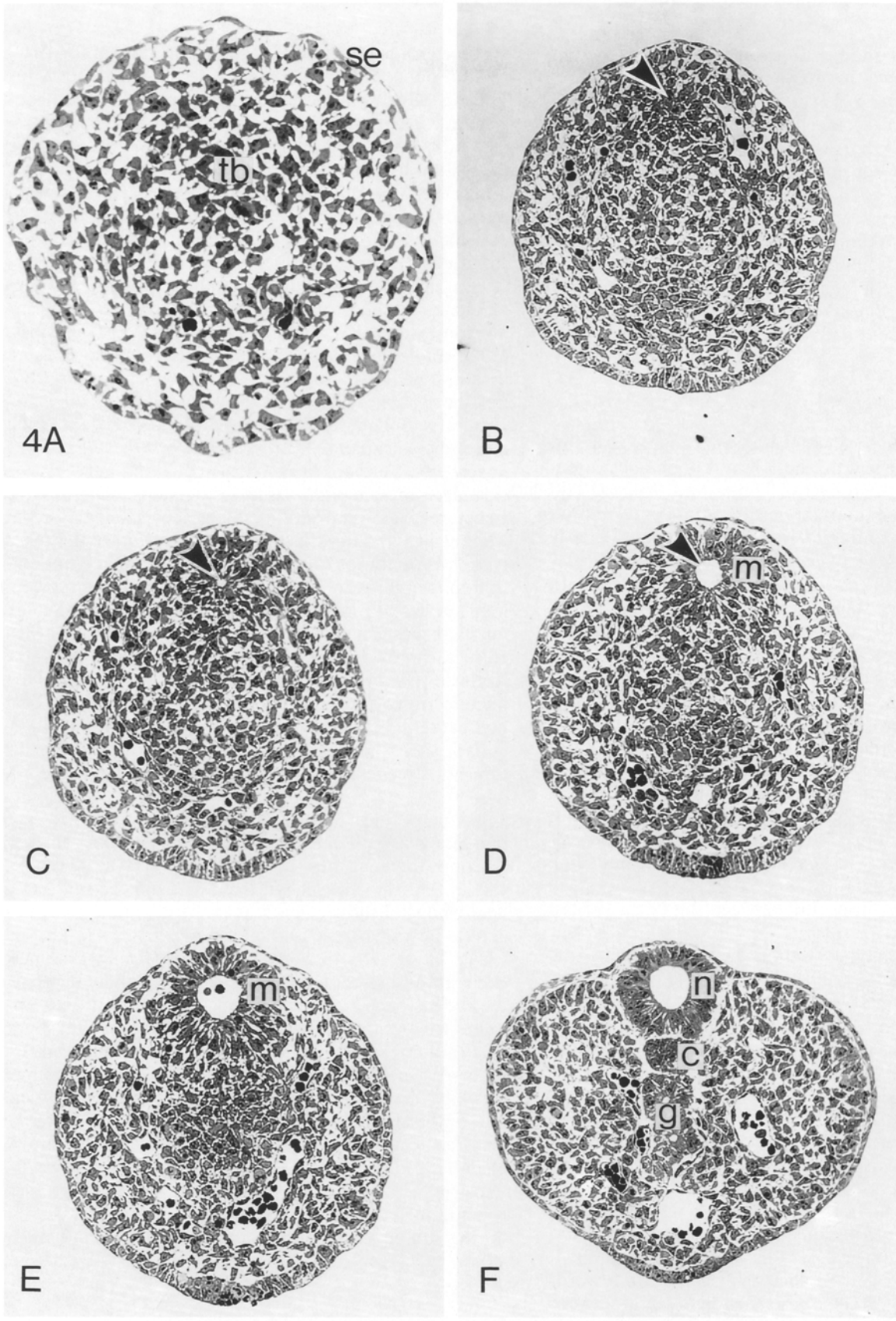


Fig. 4A–F. Transverse sections through the tail bud of a mouse embryo. They are arranged in a caudal to cranial order, showing successive stages in secondary neurulation. ×190. **A** Solid tail bud (*tb*). *se*, surface ectoderm. **B** Condensation of mesenchymal cells around a focal point (*arrowhead*) which marks the site of the secondary neurocoele in more cranial sections. **C** Appearance of lumen (*arrowhead*) surrounded by condensation of cells. **D**, **E** Sections at more cranial level, representing a more advanced stage in development. The lumen (*arrowhead*) is surrounded by radiating cells which are undergoing mesenchymal to epithelial transformation to form a neuroepithelium. These cells in transition are known collectively as a medullary rosette. **F** Section through the secondary neural tube (*n*). A notochord (*n*) and tail gut (*g*) are also present (modified with permission from Schoenwolf 1984)

a neural tube that comprises a single layer of columnar cells.

There is however no overlap zone of neurulation in lampreys as in chicks, since the entire neural tube develops by cavitation. There is no classical primary neuraxis development; the entire neural tube is formed by the hollowing out of a solid rod of cells, in a fashion similar to that in secondary neuraxial development (Weiss 1971).

There is yet another feature in the differentiating lamprey tail bud that is absent in chicks: there is an opening in the dorsum of the neural tube near its caudal limit. The significance of this opening, which is continuous with the connective tissue space, is unknown.

Remodelling of the tail bud

After the initiation of cellular differentiation, and attainment of maximum length, the tail bud undergoes extensive remodelling. A study by Tibbles and Wiley (1988) suggests that in mammals at least, the final contribution of the tail bud to different levels of the neonate budy appears to be related to whether or not the particular species has a tail. In general, the tail bud in species with reduced tails or no tails such as chickens (Constanzo et al. 1982), hamsters (Shedden and Wiley 1987) and humans (Müller and O'Rahilly 1987), gives rise to the lower lumbar, sacral and coccygeal levels of the spinal column and related axial structures. In long-tailed species, the contribution of the tail bud to the neonate axis appears to be minimal.

In chicks, the tail bud reaches its maximum length at approximately Hamburger-Hamilton (1951) stage 25 (between 4 and 5 days of incubation), after which it undergoes remodelling (Schoenwolf 1981; Mills and Bellairs 1989). According to Schoenwolf (1981), remodelling consists of three events:

1. Differential growth occurs between the tip of the tail and the more anterior regions.
2. The anterior regions become incorporated into the caudal portions of the trunk.
3. Cell death occurs at the tip of the tail.

These processes result in the loss of the distal portions of the tail (which includes the still undifferentiated tail bud mesenchyme) with the retention of the more proximal region as the definitive 'tail' in the neonate.

The observations of Schoenwolf (1981) were corroborated by the reports of Smith and McLachlan (1989) that the tail bud begins production of mitotic inhibitors at stage 18 (3 days of incubation) and continues this until stage 6 (day 4–5), the time when lengthening stops and regression begins. Conversely, high pyknotic rates were reported at stage 25 (Mills and Bellairs 1989). It has been suggested that the increasing cell death towards the tip of the tail prevents the extension of somitogenesis into the tip of the tail, resulting in a region of unsegmented mesoderm during normal development (Sanders et al. 1986; Bellairs 1986; Bellairs and Sanders 1986).

The final contribution of the tail bud to the caudal neuraxis after remodelling appears to be related to the susceptibility of that particular species to caudally localized neural tube defects/spina bifida (Hughes and Freeman 1974; Dryden 1980; Tibbles and Wiley 1988).

Histogenetic potential of the tail bud

There have been numerous attempts to define the histogenetic capacity of the tail bud. Early studies in which the entire caudal region of the chick embryo containing the tail bud was grafted onto the chorioallantoic membrane (Seevers 1932) or in a slit at the base of wing buds (Fox 1949) of homotypic host embryos suggested that the tail bud could only give rise to generalized tail structures, but not notochord or neural tube. Criley (1969), however, by extirpation of the anterior portion of chick tail buds showed that the isolated posterior portions were able to differentiate into neural tubes. Later studies by Schoenwolf and his co-workers using tritium-labelled homografts (Schoenwolf 1977), extirpation experiments (Schoenwolf 1978) and chick-quail heterografts (Schoenwolf et al. 1985) showed that the chick tail bud was capable of differentiating into neural tube, neural crest derivatives such as ganglia and nerve fibres, cartilage, muscle and vascular elements. These same structures were reported in isolated chick tail buds that were grown as grafts in the coelomic cavities of homotypic host embryos (Griffith and Wiley 1989a). In addition, however, kidney tubules were observed in the coelomic grafts. We have recently found that isolated chick tail bud mesenchyme, under appropriate conditions in culture, can give rise to a variety of cell types comparable to those seen in situ (Griffith and Sanders 1991). These include myocytes, chondrocytes, neuroepithelium and neural crest derivatives (such as melanocytes), the differentiation of which appears to be influenced by substratum adhesiveness and the extracellular matrix (see below).

As mentioned earlier, the caudalmost region of the tail bud mesenchyme, although it forms somitomeres, does not segment into somites. Krenn et al. (1990) have shown that this mesenchyme differs from the more rostral regions of the segmental plate by its failure to differentiate into myogenic and chondrogenic cells in grafted tissue. These authors therefore consider the tail bud mesenchyme as a structure distinct from the segmental plate.

The histogenetic potential of the rodent tail bud was examined by Tam (1984), who grafted mouse tail buds to the kidney capsules of adult homotypic hosts. He reported the development of neural tube, its surrounding cartilage and musculature, ganglia, nerve fibres and blood vessels. In addition, the tail bud is believed to give rise to the caudal levels of the notochord (Gajovic et al. 1989) and tail gut (Svajger et al. 1985). However, the structures developing from the tail bud in long-tailed rodents such as mice, are at levels that normally regress during subsequent fetal development (Tibbles and Wiley 1988).

Factors influencing tail bud differentiation

Changes in glycoconjugate expression

It has been well established that many of the complex cellular rearrangements and interactions of morphogenesis and differentiation are triggered by the interactions of the carbohydrate moieties of cell surface glycoproteins

Table 1. Lectin specificities

Glucose/mannose group: Inhibitors: D-Glc; D-Man	
Con A	αMan > αGlc > GlcNAc
LCA	αMan > αGlc > GlcNAc
PSA	αMan > αGlc = GlcNAc
N-acetylglucosamine group: Inhibitor: GlcNAc	
WGA	GlcNAc(β1–4GlcNAc)$_{1,2}$ > βGlcNAc > Neu5Ac
sWGA	βGlcNAc
N-acetylgalactosamine/galactose group: Inhibitors: GalNAc; D-Gal; β-lactose	
DBA	GalNAcα1–3GalNAc ≫ αGalNAc
SBA	α and βGalNAc > α and βGal
SJA	α and βGalNAc > α and βGal
BSL-1 (=GSA-1)	αGalNAc > αGal
PNA	Galβ1–3GalNAc > α and βGal
RCA-1	βGal > αGal ≫ GalNAc
PVE	α and βGalNAc
PVL	α and βGalNAc
L-fucose group: Inhibitor: L-Fuc	
UEA-1	αL-Fuc

and/or glycolipids (reviewed by Moscona 1974; Muramatsu 1988a, b; Bourrillon and Aubrey 1989; Sanders 1989). Cell-cell recognition, which often leads to the formation of selective contacts that enable cells to associate into tissues, depends largely on the expression of specific macromolecules associated with the cell surface (Moscona 1974; Edelman 1983). There is evidence that cell surface glycoproteins and their modifications during the course of cell differentiation, play fundamental roles during various phases in embryogenesis (Thiery et al. 1982; Edelman 1983; Sanders 1986a, b).

During the differentiation of the tail bud into the secondary neural tube and other caudal axial structures in chicks and in mice, there are sequential changes in the distribution of cell surface and extracellular matrix glycoconjugates, as revealed by lectin histochemistry (Griffith and Wiley 1989b, 1990a). Lectins are proteins and glycoproteins of non-immune origin that recognize and bind in an antibody-like fashion to specific sugar residues. The changes in distribution of several particular lectins show a correlation with events occurring during secondary neurulation. Table 1 shows the binding specificities of various lectins to sugar residues. The lectins, wheat germ agglutinin (WGA), peanut agglutinin (PNA), and the mannose/glucose-binding lectins, concanavalin A (Con A), *Lens culinaris* agglutinin (LCA) and *Pisum sativum* agglutinin (PSA) (Damjanov 1987), showed the most interesting binding patterns during the differentiation of the tail bud in both chick and mouse embryos. A summary is given in Tables 2–4.

In general, there was a decrease in lectin binding with increasingly advanced stages in development, as shown by serial sections through the tail bud. Since differentiation proceeds in a cranial to caudal fashion, sections viewed in a caudal to cranial sequence would represent increasingly advanced stages in development. In the undifferentiated tail bud and solid medullary cord, the lectins were homogeneously bound. At the start of differentiation and cavitation, however, except for PNA, the lectins became bound to the peripheral cells of the medullary cord. The central cells were either more lightly stained or unstained. Regions where cavities appeared during subsequent development were marked by foci of lectin binding. This pattern was especially striking in sections stained with WGA. In the case of PNA, the reverse happens during medullary cord differentiation. The central cells but not the peripheral cells retain their affinity for PNA. This may be explained by results of other studies which suggest that PNA tends to bind to developmentally and/or functionally immature cells and tissues (Aulthouse and Solursh 1987).

The changing patterns of lectin binding were more striking in chick embryos than in mouse embryos (Table 4; Griffith and Wiley 1989b), which may be in keeping with the more complex mode of secondary neurulation in chick embryos. As in the chick embryo, there was a general gradual decrease in lectin staining with

Table 2. Binding of lectins in the chick tail bud during HH stages 13–15[a]

Lectin	Solid medullary cord	Cavitating medullary cord					Secondary portion of neural tube			Notochord	
		Luminal border	Basal border	PC	CC	Ventral margin	Luminal border	Basal border	Cells	Basal border	Cells
Con A	+	−	+	+	+	++	−	+	+	−	++
LCA	++++	+++++	+++++	++++	+++	++++	+++++	+++++	++++	+++++	++++
PSA	++	+++	++	++	+	++	−	+++	++	+++	++
PVE	++	+++	+++	++	+	++	+++	−	++	−	+
RCA	+++	++++	++++	++	++	+++	+++	+++	+++	++++	+++
WGA	+++	+++	+++	++	+	++++	+++	+++	++	++++	+++
sWGA	+	+	+	−	−	+	+++	+++	−	+	+
PNA	+++	++++	++++	+	++++	++	++++	++++	+	+++	++

[a] Staining intensity is based on a subjectively estimated scale from + to +++++, with − being negative and +++++ being most positive
PC, peripheral cells; CC, central cells
From Griffith and Wiley (1990a)

Table 3. Binding of lectins in the chick tail bud during HH stages 17–19[a]

Lectin	Cavitating tail bud (caudalmost region of tail)					Mature secondary neural tube			Notochord	
	Luminal border	Basal border	PC	CC	Ventral margin	Luminal border	Basal border	Cells	Basal border	Cells
Con A	−	+	+	+	+	−	+	−	+	+
LCA	++	++++	+++	+++	+++	+++	++++	+++	++++	+++
PSA	++	+++	++	+	+++	+++	+++	++	+++	+++
PVE	+	+	−	−	−	+	+++	−	++	−
RCA	++	+++	++	++	++	++	+++	++	+++	++
WGA	++	+++	++	+	+++	++	++	+	+++	+
sWGA	+	+	−	−	−	+	+	−	+	−
PNA	++	+++	+	++	++	++	+++	−	++	−

[a] Staining intensity is based on a subjectively estimated scale from + to +++++, with − being negative and ++++ being most positive
PC, peripheral cells; CC, central cells
From Griffith and Wiley (1990a)

Table 4. Binding patterns of different lectins to the various tissues of the mouse tail bud during secondary neurulation

Lectin	Secondary neural tube			Notochord	Developing tail gut
	Cells	Luminal border	Abluminal border		
WGA	++	++++	+++	++	+
sWGA	++	++++	−	+++	++
LCA	++++	++++	++++	++++	++++
PSA	++	++	−	+++	+++
Con A	+	−	−	+	+
PNA	+	+++	+++	−	−
RCA	+	+++	+	++	+
BSL I	T	++	−	T	T
DBA	−	−	−	−	−
PVE	T	++	−	T	T
PVL	T	−	−	T	T
SBA	T	++	−	T	T
SJA	−	−	−	−	−
UEA	−	−	−	−	−

Staining intensity is based on a subjectively estimated scale from + to ++++, with − being negative and ++++ being most positive
T, trace
From Griffith and Wiley (1989b)

increasingly advanced stages in development. In addition, a small area of lectin staining present in morphogenetically homogeneous tail buds marked the position of the neurocoele that is present in more advanced stages of differentiation.

Sialoconjugates, N-CAM and tail bud development

In order to determine whether the sequential changes of glycoconjugates observed during normal development are indicative of their possible role in tail bud development, Griffith and Wiley (1990b) administered a variety of lectins to chick embryos during early stages of tail bud development, by microinjection. Specific caudal axial defects involving the neural tube and notochord were elicited by exposure of developing tail buds to the lectin WGA. The most commonly observed defects were the presence of accessory neural tube or notochord segments, and notochord-neural tube fusions. The incidence of malformation was dependent upon both the dose of WGA received and the stage of development at the time of treatment. In addition, there was a correlation between the binding of the lectin to affected tissues (as visualized using an anti-lectin antibody) and the abnormal development of the tail bud (Fig. 5).

The lectin WGA binds to sialic acid residues of glycoconjugates as well as to N-acetylglucosamine (Damjanov 1987). However, its effects on tail bud development appeared to be related to its affinity for sialic acid residues, because treatment of embryos with *Limulus polyphemus* lectin (LPL; with only sialic acid specificity) resulted in similar defects, but treatment with succinylated WGA (sWGA; with only N-acetylglucosamine specificity) or

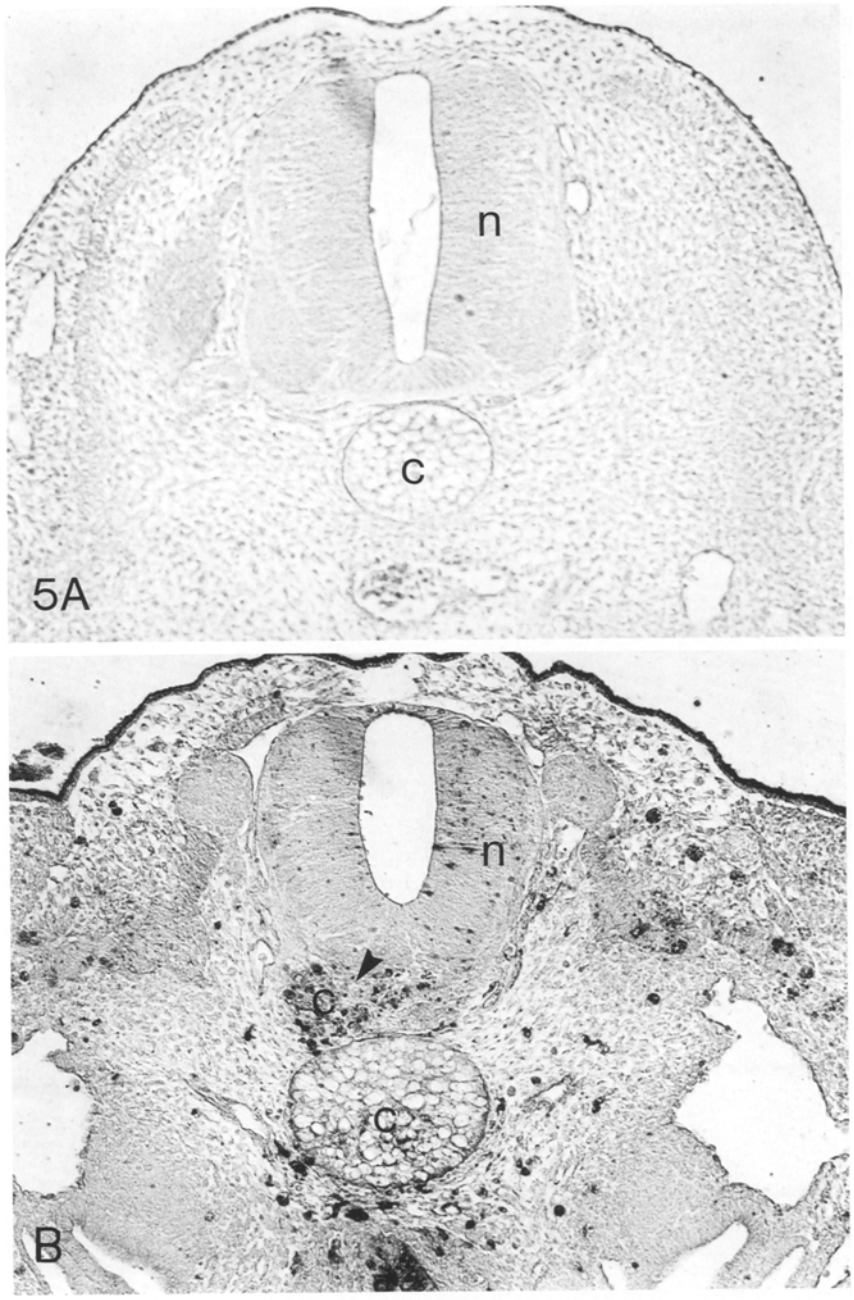


Fig. 5A, B. Sections through embryos stained with an antibody to wheat germ agglutinin (WGA). ×200. **A** Saline-treated control. *n*, neural tube; *c*, notochord. **B** Embryo previously injected with WGA. Dark spots represent areas of lectin binding. These were found in areas where defects occurred. The abnormal fusion of an accessory fragment of notochord (*c*) to the neural tube is indicated by the *arrowhead* (from Griffith and Wiley 1990b)

lectins with other sugar specificities, produced defects that differed from those of WGA or LPL, and only at much higher doses. Hence, it is most likely that the effects of WGA were due to its affinity for sialic acid residues. These results suggest an important role for glycoconjugates in general, and sialoconjugates in particular, in the differentiation of the tail bud.

As sialic acids are ubiquitous molecules, trying to associate the sialic acid residues disrupted by WGA-binding with specific molecules of the cell surface and/or extracellular matrix poses a problem. As a preliminary step in an attempt to solve this problem, Griffith and Wiley (1991) compared the sequential changes in distribution of sialic acid residues during chick secondary neurulation, as visualized using WGA, to the changes in distribution of the neural cell adhesion molecule, N-CAM, during tail bud development. N-CAM, was chosen as a possible macromolecule that was recognized and affected by WGA, because it is known to be associated with large amounts of sialic acid residues, arranged in homopolymeric chains, known as polysialic acid (PSA).

There are several forms of N-CAM. The form that is predominant in immature tissues contains up to 30% by weight of polysialic acid (Rutishauser et al. 1985; Rutishauser and Goridis 1986; Klein et al. 1988; Rutishauser et al. 1988). In the early development of the chick central nervous system, the form of N-CAM with a low PSA content is believed to maintain the integrity of the tissue under conditions of mechanical stress, e.g. folding

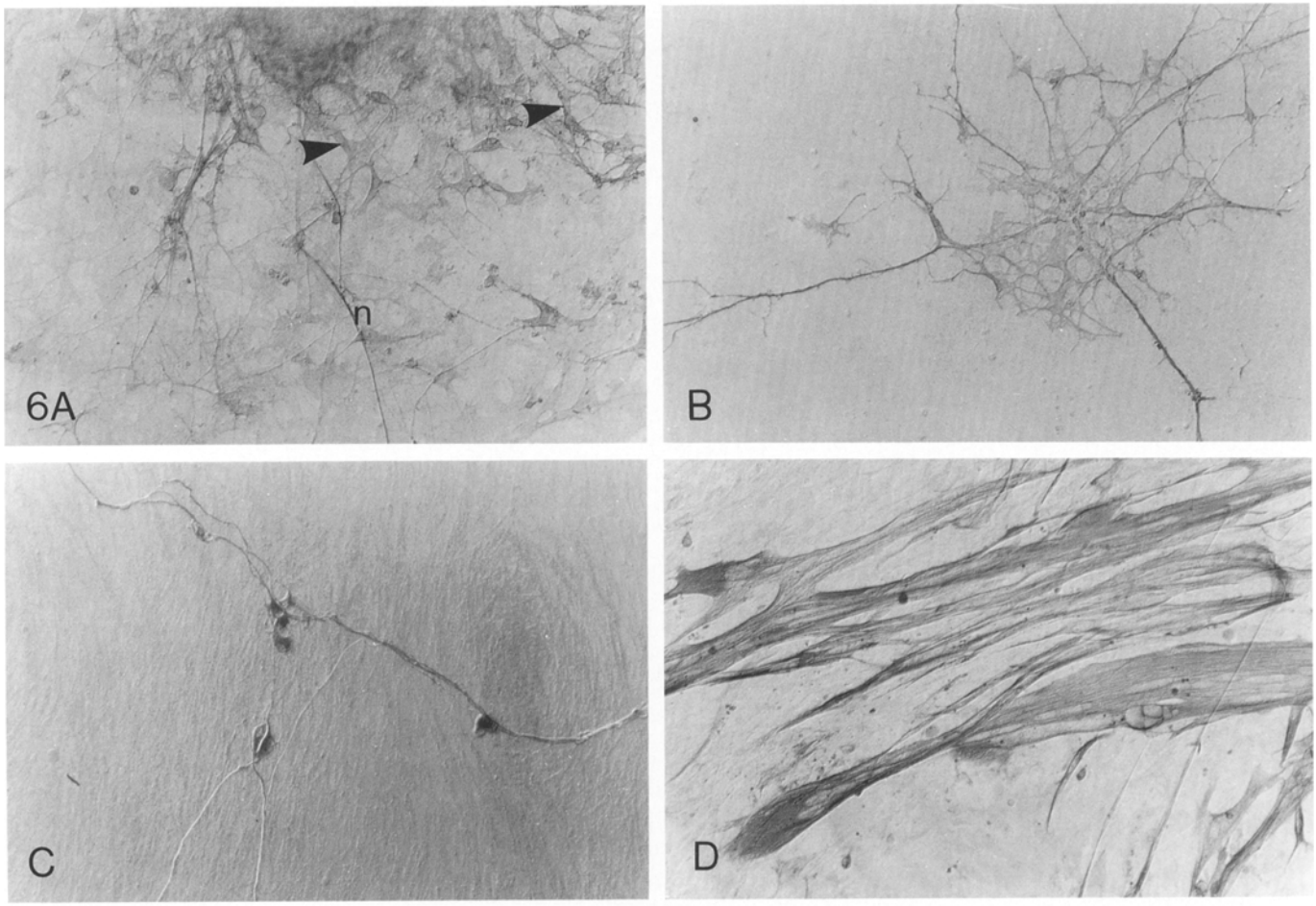


Fig. 6A–D. The effect of various ECM components, used as substrata, on the differentiation of tail bud mesenchyme in culture. **A** Explant grown on laminin for 6 days and stained for N-CAM. N-CAM-positive neurites (*n*) are present as well as elongated cells (*arrowheads*), which may represent neuroepithelial cells. ×180. **B** Explant on laminin after 6 days in culture, showing neurites positive for the HNK-1 antibody. ×180. **C** Explant after 6 days on fibronectin, showing the formation of an epithelioid sheet. Only a few neurites are present, and only in a very small proportion of cultures. ×100. **D** Tail bud mesenchyme on type I collagen, after 6 days in culture, showing myoblasts stained with an anti-desmin antibody. ×180 (see Griffith and Sanders 1991)

of the neural plate to form primary neural tube (Sunshine et al. 1987). This is achieved by increasing cell-cell contact through homophilic binding of N-CAM molecules on one cell to those on a neighbouring cell, thus facilitating any further interactions between the cells (Rutishauser et al. 1988). The highly sialylated form of N-CAM, on the other hand, prevents membrane apposition and cell-cell contact by steric hinderance provided by its long PSA chains. This would allow cells to undergo morphogenetic movements and other rearrangements which require flexibility (Sunshine et al. 1987; Rutishauser et al. 1988).

Similarities in the distribution of WGA-bound sialic acid residues (Griffith and Wiley 1990b) and N-CAM molecules (Griffith and Wiley 1991) were found in differentiating chick tail buds at various stages. In both cases, staining with the lectin and anti-N-CAM antibody was more evident in the peripheral cells of the differentiating medullary cord than in the central cells. The apices of peripheral cells which make up the linings of lumina that form during cavitation of the medullary cord were also intensely stained by both the lectin and the antibody.

Administration of WGA, LPL or a polyclonal anti-N-CAM antibody by sub-blastodermal injection under the tail bud, induced morphologically comparable malformations of the caudal axis. Administration of either the lectin or anti-N-CAM antibody induced the presence of accessory neural tube lumina, abnormal branching of the notochord, abnormal neural tube-notochord fusions and projections of neural tube and/or notochord tissues into the gut (ourenteric outgrowths). While the results of this study do not prove that N-CAM is involved in the development of the secondary neural tube and other associated tail bud-derived structures, they nevertheless show correlations between the distribution of WGA-binding sialic acid residues and N-CAM molecules during tail bud differentiation, and also the development of comparable caudal axial defects in response to either administration of WGA or an anti-N-CAM

antibody as a teratogen during tail bud development. This leaves open the possibility that N-CAM may be involved in secondary neurulation.

According to Rutishauser et al. (1988), the PSA portion of the N-CAM molecule on its own can regulate cellular interactions that involve molecules other than N-CAM. For example, in neurite outgrowth from the dorsal root ganglion, removal of PSA from the N-CAM molecule with an endoneuraminidase, endo-N, resulted in increased fibre-fibre adhesion (Rutishauser et al. 1988). Comparison of the distribution of PSA with that of the whole N-CAM molecule showed that at early stages of tail bud differentiation (HH stages 13–15), N-CAM was localized to the apices of peripheral cells that form the borders of the lumina during cavitation, while antibody binding to PSA was minimal. The use of unspecified polyclonal antibody showed localization of N-CAM to the ventral horn and floor plate of the mature secondary neural tube, while the use of a monoclonal antibody against N-CAM containing long chains of PSA, the 5A5 antibody, showed localization primarily to the floor plate (Griffith and Wiley 1991). It is of some interest to note that the floor plate area was the portion of the neural tube that was always involved in neural tube-notochord fusions, or was completely replaced by a notochord segment.

Effects of extracellular matrix components

The macromolecules of the extracellular matrix are important factors in embryonic differentiation, and have been demonstrated to exert profound effects in many systems (Hay 1981; Sanders 1989). As the extracellular matrix has been known to maintain the differentiated state of various epithelia (Beck et al. 1990), we tested the ability of its various components to support the mesenchymal to epithelial transformation of tail bud mesenchymal cells into neuroepithelium in culture (Griffith and Sanders 1991). Several of the matrix components tested were found to favour the differentiation of cell types distinct from the morphologically homogeneous mass of explanted tail bud mesenchymal cells. The effects of the different substrata used are summarized in Tables 5 and 6.

Laminin, which is a glycoprotein and major component of the basement membrane, mediates cellular attachment and maintains the differentiated state of epithelial and endothelial cells (Beck et al. 1990). It is also one of the most potent promoters of neurite outgrowth (Manthorpe et al. 1983; Rogers et al. 1983; Edgar et al. 1984, 1988). In our cultures, laminin, apart from supporting extensive fibroblastic outgrowth from tail bud mesenchyme, also promoted the differentiation of neuroepithelium, neural crest derivatives (neurons, melanocytes) and mesoderm derivatives (muscle). Matrigel (basement membrane gel, Collaborative Research), which contains mainly laminin and type IV collagen, had the same effect as laminin. In addition, however, chondrogenic foci were found in several tail bud explants cultured on Matrigel.

Table 5. Epithelial vs mesenchymal differentiation of tail bud mesenchyme cells explanted onto various ECM components as substrata

ECM component	Tail bud derivatives		
	Fibroblasts	Epithelium	Neuro-epithelium
Fibronectin	−	+	+/−
Laminin	+	−	+
Matrigel	+	−	+
Collagen I	+	−	−
Collagen IV	+	−	−

+, cell type present; −, absent

Table 6. Effect of ECM components in the promotion of differentiation into mesodermal and neural crest derivatives

ECM component	Mesodermal derivatives		Neural crest derivatives	
	Muscle	Cartilage	Neurons	Melano-cytes
Fibronectin	+	−	+/−	−
Laminin	+	−	+	+
Matrigel	+	+	+	+
Collagen I	+	+	−	−
Collagen IV	+	−	+	−

+, cell type present; −, absent

The ability of these cultures to differentiate into myogenic and chondrogenic cells, argues in favour of the possibility that the tail bud mesenchyme initially contains such cells but they are subsequently lost in situ, since grafted tail bud tissue does not give rise to muscle or cartilage (Krenn et al. 1990).

Fibronectin is a cell attachment glycoprotein that is found in the extracellular matrix. On fibronectin, the tendency was for outgrowing cells to acquire an epithelioid morphology.

Collagens, types I and IV, promoted myogenesis but not neurite outgrowth. In addition, type I collagen, which is an interstitial collagen, also promoted chondrogenesis. Type IV collagen, which is located only in basement membranes, however, did not support formation of chondrogenic foci.

The development of the tail bud in culture therefore compares favourably with that in situ, and is greatly influenced by components of the extracellular matrix.

Concluding remarks

We have shown that the tail bud mesenchyme, by virtue of its pluripotent nature, can provide a valuable and accessible model for the study of cell differentiation. Its pluripotency is demonstrated by both in vivo grafting techniques and in vitro culture by its remarkable ability

to differentiate into cell and tissue types that elsewhere in the body are derived from the three classical germ layers. The pluripotent nature of this tissue may be related to its origins from the primitive streak and Hensen's node, and as such, it may afford insights into the mechanisms of the epithelial-to-mesenchymal and mesenchymal-to-epithelial cell transformations that characterize early development.

The development of the neural tube in cyclostomes (considered to be evolutionarily ancient), by hollowing out of a solid rod of cells, suggests that the process of secondary neuraxial development from the tail bud in other vertebrate classes may represent the remnants of a process that has been spared by evolution.

Acknowledgements. We would like to thank Dr. G.C. Schoenwolf, Dept. of Anatomy, University of Utah, and Wiley-Liss (publishers) for permission to reprint his micrographs; and Dr. E.W. Larsen, Dept. of Zoology, University of Toronto, for her constructive comments on the manuscript. Our research was supported by operating grants to MJW and EJS and a post-doctoral fellowship to CMG, from the Medical Research Council of Canada.

References

Aulthouse A, Solursh M (1987) The detection of a precartilage, blastema specific marker. Dev Biol 120:377–384

Beck K, Hunter I, Engel J (1990) Structure and function of laminin: anatomy of a multidomain glycoprotein. FASEB J 4:148–160

Bellairs R (1986) The tail bud and cessation of segmentation in the chick embryo. In: Bellairs R, Ede DA, Lash JW (eds) Somites in developing embryos. Plenum Press, New York, pp 161–178

Bellairs R, Sanders EJ (1986) Somitomeres in the chick tail bud: an SEM study. Anat Embryol 175:235–240

Bourrillon R, Aubery M (1989) Cell surface glycoproteins in embryonic development. Int Rev Cytol 116:257–338

Constanzo R, Watterson RL, Schoenwolf GC (1982) Evidence that secondary neurulation occurs autonomously in the chick embryo. J Exp Zool 219:233–240

Coulombre AJ, Coulombre JL (1958) The role of mechanical factors in brain morphogenesis. Anat Rec 130:289–290

Criley BB (1969) Analysis of the embryonic sources and mechanisms of development of posterior levels of chick neural tubes. J Morphol 128:465–501

Damjanov I (1987) Biology of disease. Lectin cytochemistry and histochemistry. Lab Invest 57:5–20

Desmond ME, Jacobson AG (1977) Embryonic brain enlargement requires cerebrospinal fluid pressure. Dev Biol 57:188–198

Deuchar EM (1975) Regeneration of the tail bud in *Xenopus* embryos. J Exp Zool 192:381–390

Dryden RJ (1980) Spina bifida in chick embryos: ultrastructure of open neural defects in the transitional region between primary and secondary modes of neural tube formation. In: Persaud TVN (ed) Neural and behavioral teratology, vol 4. University Park Press, Baltimore, pp 75–100

Edelman GM (1983) Cell adhesion and morphogenesis: the regulator hypothesis. Proc Natl Acad Sci USA 81:1460–1464

Edgar D, Timpl R, Thoenen H (1984) The heparin-binding domain of laminin is responsible for its effects on neurite outgrowth and neuronal survival. EMBO J 3:1463–1468

Edgar D, Timpl R, Thoenen H (1988) Structural requirements for the stimulation of neurite outgrowth by two variants of laminin and their inhibition by antibodies. J Cell Biol 106:1299–1306

Fox MH (1949) Analysis of some phases of melanoblast migration in the Barred Plymouth Rock embryos. Physiol Zool 22:1–22

Gajovic S, Kostovic-Knezevic L, Svajger A (1989) Origin of the notochord in the rat embryo tail. Anat Embryol 179:305–310

Griffith CM, Sanders EJ (1991) Effects of extracellular matrix components on the differentiation of chick embryo tail bud mesenchyme in culture. Differentiation (in press)

Griffith CM, Wiley MJ (1989a) Direct effects of retinoic acid on the development of the tail bud in chick embryos. Teratology 39:261–275

Griffith CM, Wiley MJ (1989b) Distribution of cell surface glycoconjugates during mouse secondary neurulation. Anat Embryol 180:567–575

Griffith CM, Wiley MJ (1990a) Distribution of cell surface glycoconjugates during chick secondary neurulation. Anat Rec 226:81–90

Griffith CM, Wiley MJ (1990b) Sialoconjugates and development of the tail bud. Development 108:479–489

Griffith CM, Wiley MJ (1991) NCAM, polysialic acid and tail bud development. Anat Embryol 183:205–212

Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. J Morphol 88:49–92

Hay ED (1981) Collagen and embryonic development. In: Hay ED (ed) Cell biology of extracellular matrix. Plenum Press, New York, pp 379–409

Holmdahl DE (1925a) Experimentelle Untersuchungen über die Lage der Grenze zwischen primärer und sekundärer Körperentwicklung beim Huhn. Anat Anz 59:393–396

Holmdahl DE (1925b) Die erste Entwicklung des Körpers bei den Vögeln und Säugetieren, inkl. dem Menschen, besonders mit Rücksicht auf die Bildung des Rückenmarks, des Zoloms und der entodermalen Kloake nebst einem Exkurs über die Entstehung der Spina bifida in der Lumbosakralregion. I. Gegenbaurs Morphol Jahrb 54:333–384

Holmdahl DE (1925c) Die erste Entwicklung des Körpers bei den Vögeln und Säugetieren, inkl. dem Menschen, besonders mit Rücksicht auf die Bildung des Rückenmarks, des Zoloms und der entodermalen Kloake nebst einem Exkurs über die Entstehung der Spina bifida in der Lumbosakralregion. II–V. Gegenbaurs Morphol Jahrb 55:112–208

Hughes AF, Freeman RB (1974) Comparative remarks on the development of the tail cord among higher vertebrates. J Embryol Exp Morphol 32:335–363

Jacobson AG (1988) Somitomeres: mesodermal segments of vertebrate embryos. Development 104:209–220

Karfunkel P (1974) The mechanisms of neural tube formation. Int Rev Cytol 38:245–271

Klein G, Langegger M, Goridis C, Ekblom P (1988) Neural adhesion molecules during embryonic induction and development of the kidney. Development 102:749–761

Klika A, Jelinek R (1969) The structure of the end bud and tail bud of the chick embryo. Folia Morphol 17:29–40

Krenn V, Ostermayer H, Wachtler F (1990) On the histogenetic potency of the tailbud mesoderm. Anat Embryol 181:595–601

Lemire RJ (1969) Variations in the development of the caudal neural tube in human embryos (Horizons XIV-XXI). Teratology 2:361–370

Lemire RJ, Loeser JD, Leech RW, Alvord EC Jr (1975) Secondary caudal neural tube formation. In: Normal and abnormal development of the human nervous system. Harper & Rowe, Hagerstown, pp 54–69

Manthorpe M, Engvall E, Rouslahti E, Longo FM, Davies GE, Varon S (1983) Laminin promotes neurite regeneration from cultured peripheral and central neurons. J Cell Biol 97:1882–1890

Mills CL, Bellairs R (1989) Mitosis and cell death in the tail of the chick embryo. Anat Embryol 180:301–308

Moscona AA (1974) Surface specification of embryonic cells: lectin receptors, cell recognition and specific cell ligands. In: Moscona AA (ed) The cell surface in development. Wiley, New York, pp 67–99

Müller F, O'Rahilly R (1987) The development of the human brain, the closure of the caudal neuropore, and the beginning of secondary neurulation at stage 12. Anat Embryol 176:413–430

Muramatsu T (1988a) Developmentally regulated expression of cell surface carbohydrates during mouse embryogenesis. J Cell Biochem 36:1–14

Muramatsu T (1988b) Alterations of cell surface carbohydrates during differentiation and development. Biochimie 70:1587–1596

Nakao T, Ishizawa A (1984) Light- and electron-microscopic observations of the tail bud of the larval lamprey (*Lampetra japonica*), with special reference to neural tube formation. Am J Anat 170:55–71

Rogers SL, Letourneau PC, Palm SL, McCarthy J, Furcht LT (1983) Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. Dev Biol 98:212–220

Rutishauser U, Goridis C (1986) NCAM: the molecule and its genetics. Trends Genet 2:72–76

Rutishauser U, Watanabe M, Silver J, Troy FA, Vimr ER (1985) Specific alteration of NCAM-mediated cell adhesion by an endoneuraminidase. J Cell Biol 101:1842–1849

Rutishauser U, Acheson A, Hall AK, Mann DM, Sunshine J (1988) The neural cell adhesion molecule (NCAM) as a regulator of cell-cell interactions. Science 240:53–57

Sanders EJ (1986a) Mesoderm migration in the early chick embryo. In: Browder L (ed) Developmental biology, vol 2. Plenum Press, New York, pp 449–480

Sanders EJ (1986b) Cytochemistry of the cell surface and extracellular matrix during early embryonic development. Prog Histochem Cytochem 16:1–57

Sanders EJ (1989) The cell surface in embryogenesis and carcinogenesis. Telford Press, New Jersey

Sanders EJ, Khare MK, Ooi VC, Bellairs R (1986) An experimental and morphological analysis of the tail bud mesenchyme of the chick embryo. Anat Embryol 174:179–185

Schoenwolf GC (1977) Tail (end) bud contributions to the posterior region of the chick embryo. J Exp Zool 201:227–246

Schoenwolf GC (1978) Effects of complete tail bud extirpation on early development of the posterior region of the chick embryo. Anat Rec 192:289–296

Schoenwolf GC (1979) Histological and ultrastructural observations of tail bud formation in the chick embryo. Anat Rec 193:131–148

Schoenwolf GC (1981) Morphogenetic processes involved in the remodelling of the tail region of the chick embryo. Anat Embryol 162:183–197

Schoenwolf GC (1984) Histological and ultrastructural studies of secondary neurulation in the mouse embryo. Am J Anat 169:361–376

Schoenwolf GC, DeLongo J (1980) Ultrastructure of secondary neurulation in the chick embryo. Am J Anat 158:43–63

Schoenwolf GC, Smith JL (1990) Mechanisms of neurulation: traditional viewpoint and recent advances. Development 109:243–270

Schoenwolf GC, Chandler NB, Smith JL (1985) Analysis of the origins and early fates of neural crest cells in caudal regions of avian embryos. Dev Biol 110:467–479

Seevers CH (1932) Potencies of the end bud and other caudal levels of the early chick embryo, with special reference to the origin of the metanephros. Anat Rec 54:217–246

Shedden PA, Wiley MJ (1987) The structure of secondary neurulation in golden Syrian hamsters (*Mesocricetus auratus*). Anat Rec 219:180–185

Smith J, McLachlan JC (1989) Developmental pattern of growth factor production in chick embryo limb buds. J Anat 165:159–168

Sunshine J, Balak K, Rutishauser U, Jacobson M (1987) Changes in neural cell adhesion molecule (NCAM) structure during vertebrate neural development. Proc Natl Acad Sci USA 84:5986–5990

Svajger A, Kostovic-Knezevic L, Bradamante Z, Wrischer M (1985) Tail gut formation in the rat embryo. Roux's Arch Dev Biol 194:429–432

Tam PPL (1984) The histogenetic capacity of tissues in the caudal end of the embryonic axis of the mouse. J Embryol Exp Morphol 82:253–266

Thiery JP, Duband JL, Rutishauser U, Edelman GM (1982) Cell adhesion molecules in early chicken embryogenesis. Proc Natl Acad Sci USA 79:6737–6741

Tibbles L, Wiley MJ (1988) A comparative study of the effects of retinoic acid given during the critical period for inducing spina bifida in mice and hamsters. Teratology 37:113–125

Weiss P (1971) Nervous System. In: Willier BH, Weiss PA, Hamburger V (eds) Analysis of development. Hafner, New York, pp 346–401